Transcriptional analysis of diesel exposure

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Monday night football

Cowboys 21
Redskins 18

Experimental Plan

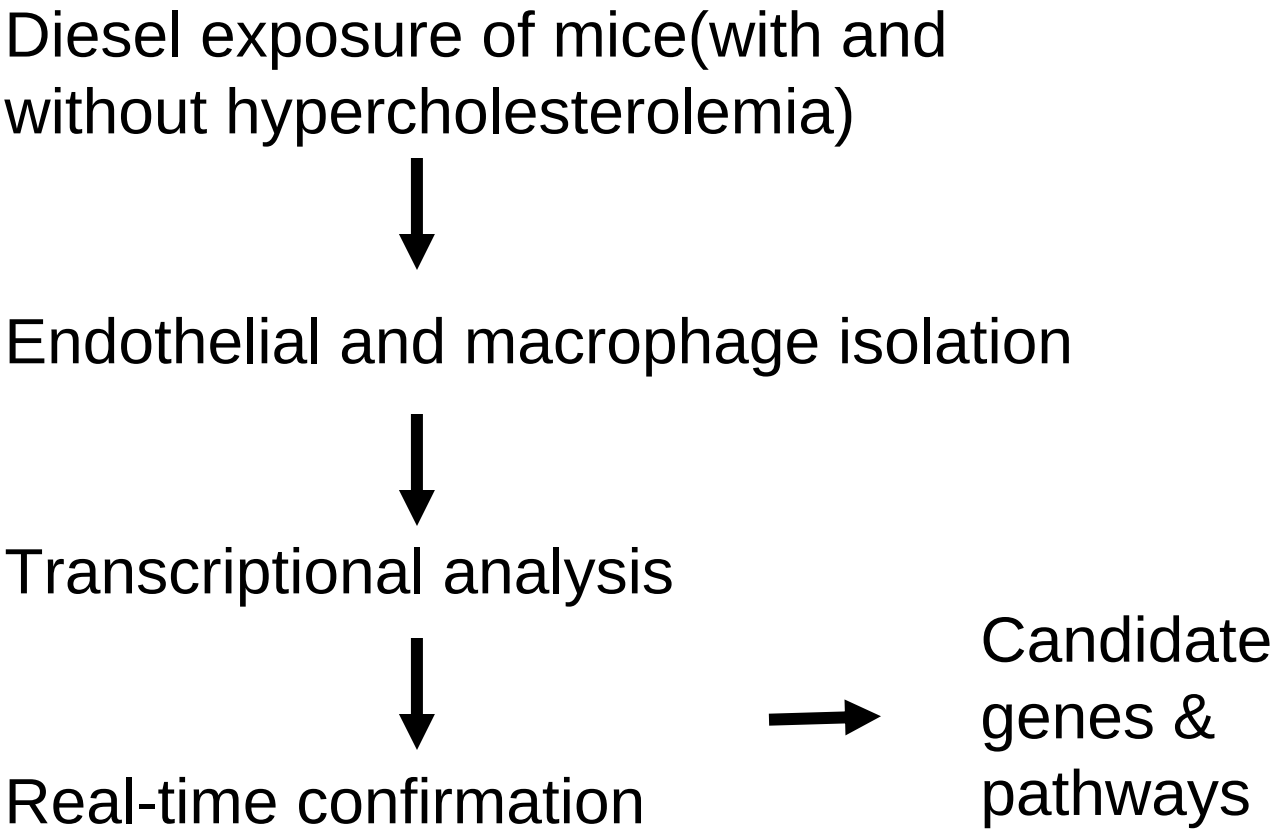
Hypothesis:

particulate-induced gene regulation in endothelial cells and macrophages will implicate the pathways that contribute to pollution-related atherogenesis.

Strategy:

thorough transcriptional analysis of in vivo gene regulation by diesel exhaust.

Experimental Plan



Diesel exposure

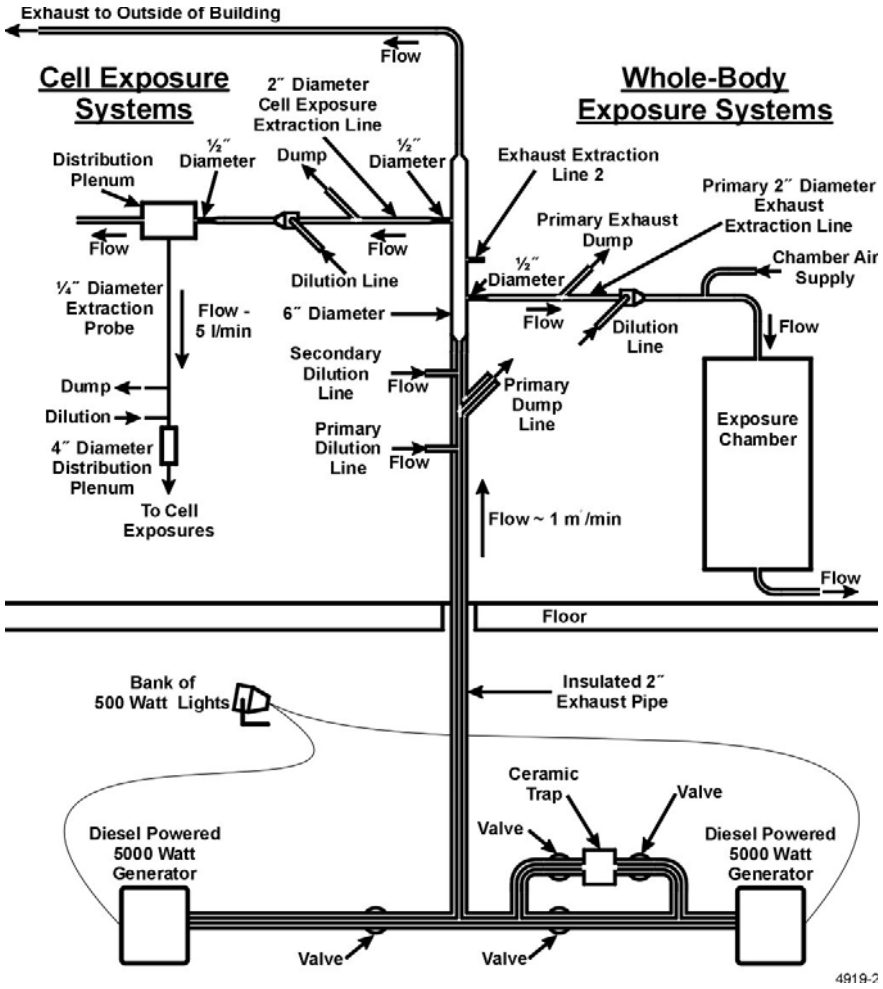
Matt Campen

Matt Reed

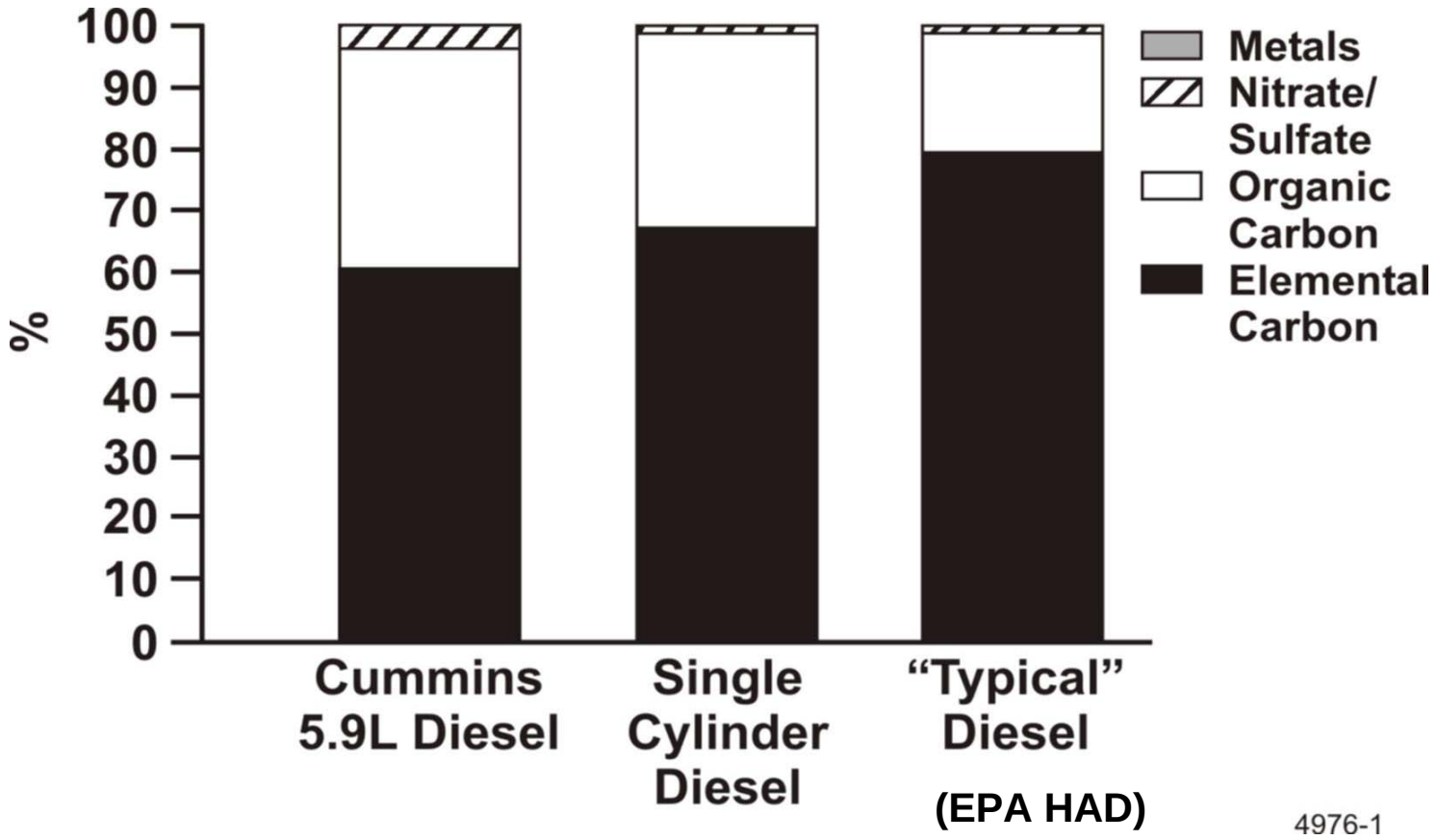
single cylinder, 5500 Watt, Yanmar diesel engine generator using nationally certified diesel fuel

Lovelace Respiratory Research Institute Albuquerque,NM

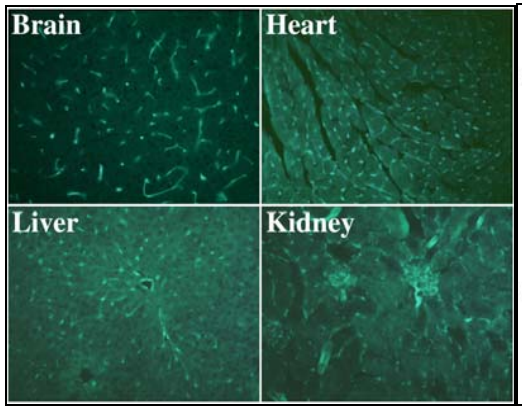
Small diesel exposure system

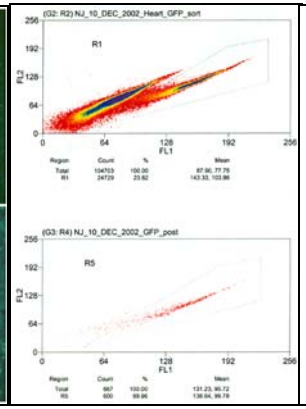


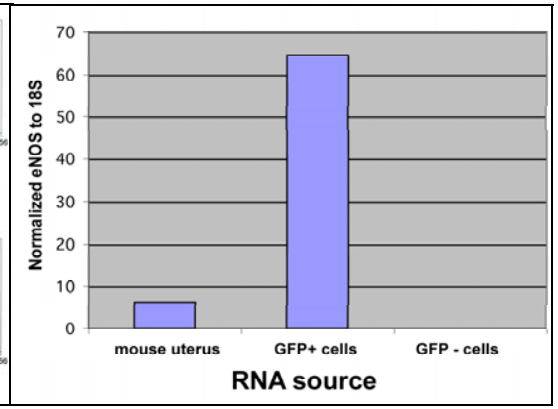
Comparison of particle compositions



Endothelial Isolation







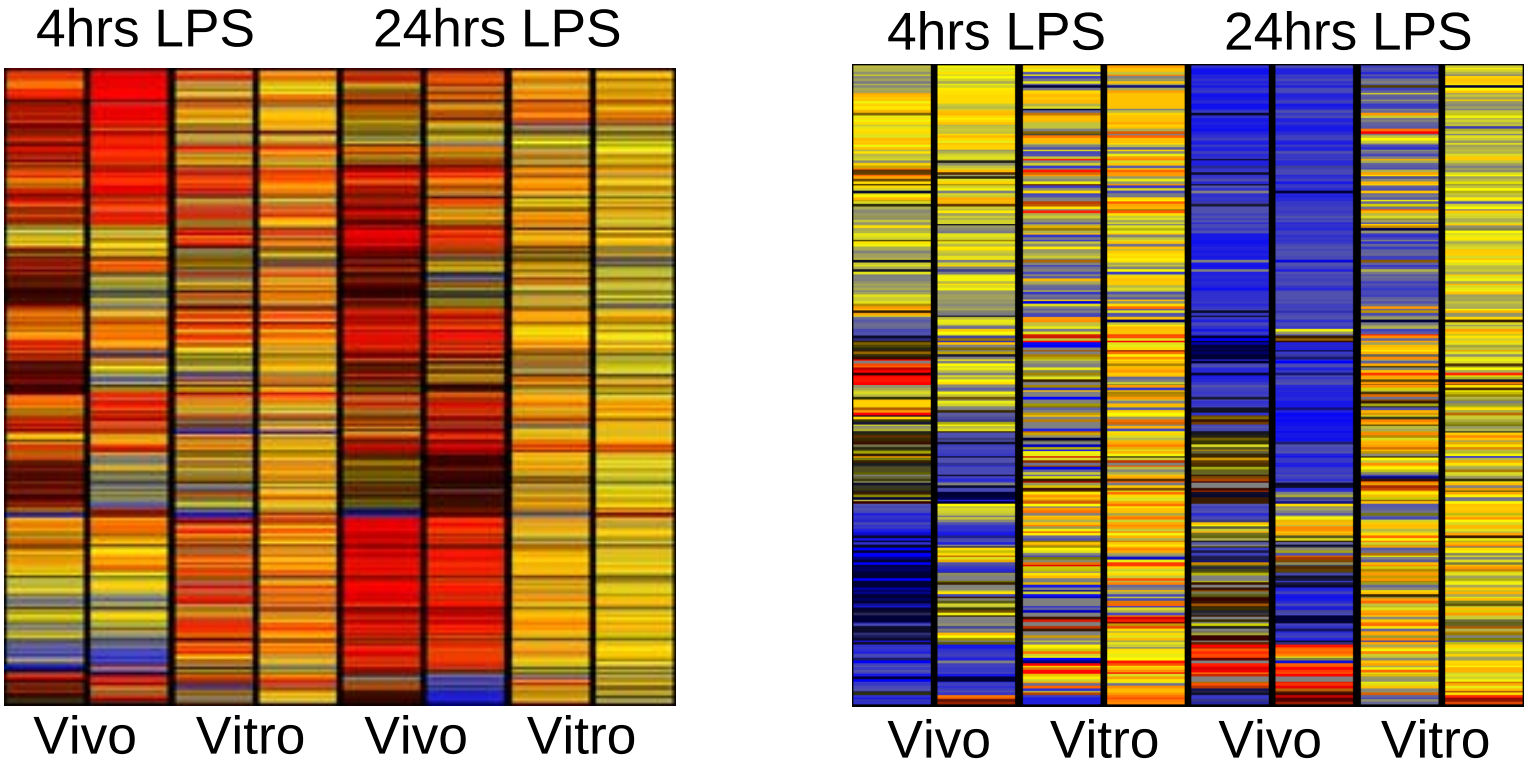
Histology
Expressed in all tissues

FACS
High purity prep

RT-PCR
Recovery is good

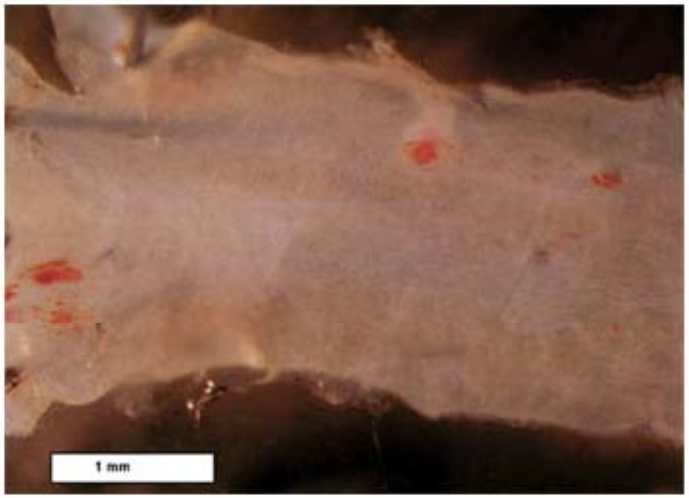
TIE2-GFP strain

In vivo gene regulation is more robust than in vitro

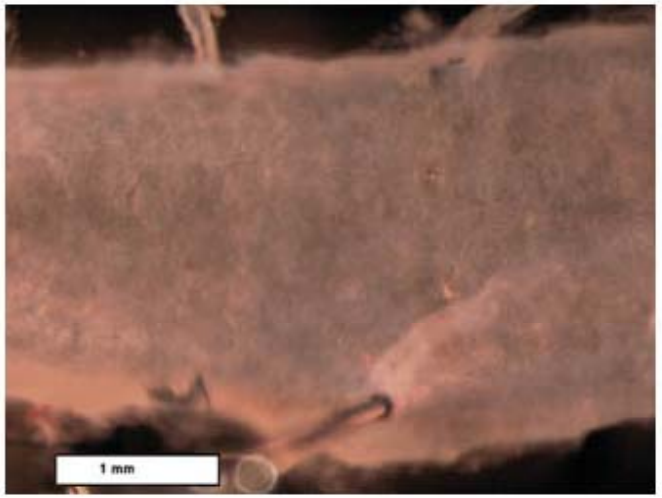


Regulated endothelial gene expression in response to equivalent doses of lipopolysaccharide

Mouse model



ApoE Knockout



Wild-type

4 wks of high fat diet

Array analysis

Combination of Operon V3 and MGH 70-mer sets

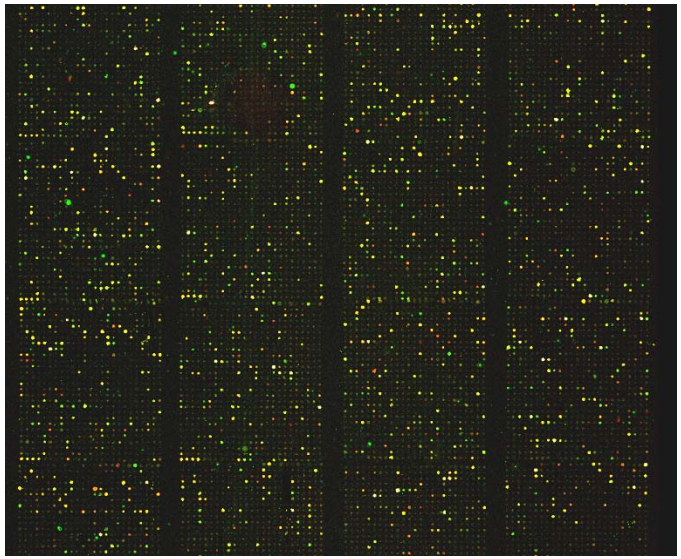
<u>advantages</u>

representation

redundancy

multiple sampling

inexpensive



1/4th of the V3 set

Specific attributes of our project

1. **Diesel exposure** - will approximate real-life exposures with effects determined in vivo.

2. **Mouse models** - allow control over dose, duration, and additional risk factors (hypercholesterolemia).

3. **Transcriptional analysis** - deeply representative, confirmation by redundancy, real-time, and technical and biological repetition.

A bit of data - protocol

Mice were exposed to 550 µg/m^3 of diesel engine exhaust for 6hr/day for 3 consecutive days.

Endothelial cells of aortas were obtained by dissection, collagenase digestion, and FACS.

After T7 amplification we compared the RNA from control and exposed cells using long oligo microarrays to identify genes that were regulated by diesel exposure.

Initial data - 3 days of diesel exposure

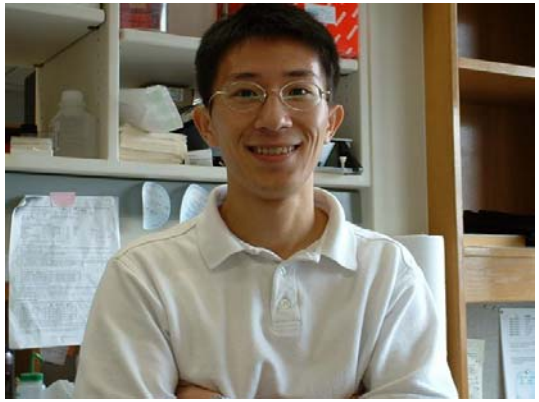
Category	Gene Name	Fold-change	Possible relevance to endothelial response to diesel exhaust
Up	Cytochrome c oxidase subunit VIII b	5	Final stage of mitochondrial electron transport
	Meso1	3	Organismal development
	Rgc32	3	Induced by sub-lytic complement activation, associated with cdc2 kinase
	Hyaluronidase 2	2.5	Endothelial glycocalyx thinned by oxidized LDL
	Timp 4	4	Tissue inhibitor of metalloproteinase
	Heme oxygenase	3	Hypoxic responses
Down	Serpin a1a	3	Distant homologue of alpha 1 antitrypsin, inhibitor of proteases
	Cyclophilin A	2.5	Released during endothelial inflammation, protein folding
	Tra1, gp96	2	Molecular chaperone
	Ccl8, chemokine receptor ligand 8	3.5	Inflammation
	Akrla4	2.5	Aldehyde reductase
	ceruloplasmin precursor	3	Copper and iron homeostasis
	atx1	3	antioxidant protein 1, metal response

RGC-32, a modifier of cyclin activity known to increase cell cycle progression in vascular smooth muscle

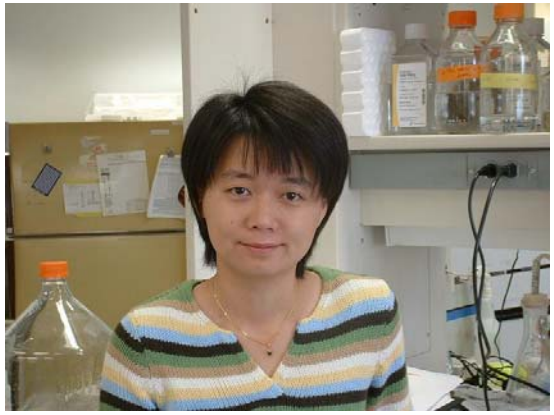
aldehyde reductase, an enzyme with a potential role in elimination of atherogenic aldehydes generated in response to oxidized LDL

Diesel investigators

Greg Maresh



Huaxia Xu



Ling Zhang